Abscisic Acid Levels and Metabolism in the Leaf Epidermal Tissue of *Tulipa gesneriana* L. and *Commelina communis* L.

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**Abstract.** We have shown the presence of abscisic acid (ABA) in abaxial epidermal strips taken from *Tulipa gesneriana* and *Commelina communis* and that the ABA level rises in the epidermis when leaves are water stressed. ABA levels had risen 50% in the abaxial epidermis of *C. communis* 30 min after the leaves lost 10% of their fresh weight. Epidermis from both *T. gesneriana* and *C. communis* metabolize [14C]ABA to several products probably including phaseic acid (PA) and dihydrophaseic acid (DPA).

**Key words:** Abscisic acid - *Commelina* - Leaf epidermal tissue - Radioimmunoassay (ABA) - *Tulipa* - Water stress.

**Introduction**

In recent years considerable evidence has been obtained which suggests that ABA regulates water loss from mesophytic plants under stress conditions by reducing stomatal aperture (Milborrow, 1974; Raschke, 1975). One line of evidence for this suggestion is that ABA levels rise rapidly and dramatically in leaves when either whole plants or individual leaves are water stressed (Milborrow, 1974). Although the inference has been drawn that ABA affects the guard cells directly (Raschke, 1975), there is no published evidence that guard cells contain ABA and the first report that the epidermis contains ABA whose level rises when the leaves are stressed has only recently appeared (Loveys, 1977).

We have been interested in the kinetics of ABA rise in water-stressed leaves (Walton et al., 1977a). This report extends our observations to include epidermal tissue of *Commelina communis* and also describes the metabolism of [14C]ABA in epidermal tissue of *Commelina communis* and *Tulipa gesneriana*.

**Materials and Methods**

1. **Kinetic Experiments**

*Commelina communis* plants were grown in soil in the greenhouse for 10 to 12 weeks and transferred to a growth chamber (22 ± 1 °C, continuous light at 40 W m⁻²) the day before use. For each treatment, 15 leaves were randomly selected in batches of 5 from 25 plants growing in 5 pots. The 3 youngest fully expanded leaves were used from each plant. Leaves to be stressed were detached and allowed to lose 10% of their fresh weight which required about 10 min. The stressed leaves were wrapped in aluminum foil and kept at 22 °C for appropriate periods of time. Non-stressed leaves were either immediately wrapped in foil or extracted after detachment. The various treatments were replicated 3 to 5 times. Abaxial epidermis was stripped from the leaves and both the strips and portions of the leaves without abaxial epidermis were frozen with dry ice. The stripping time for each batch of 5 leaves took about 5 min.

**Tissue was extracted in 2 different ways.**

**Method 1:** The frozen tissue was homogenized with 80% acetone, the homogenate was filtered and the residue dried at 60°C for 24 h and weighed. Chlorophyll determinations (Bruinsma, 1961) were done on the filtrate which was then evaporated with a N₂ stream. The residue was taken up in 10 mM NaPO₄, pH 7.3, and used for radioimmunoassay (RIA).

**Method 2:** The frozen tissue was added to water-saturated ethyl acetate and kept at 4°C for 4 days. The extract was dried at 60°C and weighed and the ethyl acetate solution partitioned against 1% NaHCO₃. The NaHCO₃ solution was then treated as in Method 1.

2. **ABA Determinations by Gas Chromatography** (GC)

*T. gesneriana*, cv. Christmas Marvel, and *C. communis* were grown in the greenhouse for 8 to 10 weeks. The detached leaves were stressed by a 10% fresh weight loss.

**Abbreviations:** ABA = abscisic acid; RIA = radioimmunoassay; PA = phaseic acid; DPA = dihydrophaseic acid; TLC = thin-layer chromatography; GC = gas chromatography
Leaf and abaxial epidermal tissues were homogenized in 80% acetone with a mortar and pestle after which 30,000 d min$^{-1}$ [3H]ABA (sp act 7.9 x 10$^{14}$ Bq/mmol) was added to determine purification losses. The homogenates were filtered and the filtrate evaporated in vacuo. The residue was taken up in 1% NaHCO$_3$ and partitioned 3 times with chloroform. The aqueous phase was adjusted to pH 2.5 with HCl and partitioned 3 times with methylene chloride. The methylene chloride was removed with N$_2$ and the residue methylated with diazomethane. The ABA methyl ester was purified by thin-layer chromatography (TLC) on 0.25 mm thick E. Merck (Darmstadt, DRG) Silica gel F-254 plates and further purified by high performance liquid chromatography using a 3 x 250 mm column packed with 5 μm Spherisorb silica (Spectra Physics, Piscuitaway, NJ, USA). Elution was isocratic with hexane:isopropanol (96:4, v/v). ABA methyl ester was estimated by GC using an electron-capture detector (Harrison and Walton, 1975).

3. $[^{14}C]$ABA Metabolism

Epidermal strips (0.25-0.35 g fr wt) were incubated in 50 ml Erlenmeyer flasks in 3 ml containing 10 mM HEPES, pH 6.0, 150 μg chloramphenicol, 20 mM CaCl$_2$ and 5.7 x 10$^3$ Bq 2-$[^{14}C]$(RS)-ABA (5 Ci mol$^{-1}$). For uptake into whole leaves, petioles were placed in vials with 0.15 ml solution containing 10 mM HEPES, pH 6.0, (5 Ci- mol$^{-1}$). For uptake into whole leaves, petioles were placed on the plates and further purified by high performance liquid chromatography using a 3 x 250 mm column packed with 5 μm Spherisorb silica (Spectra Physics, Piscuitaway, NJ, USA). Elution was isocratic with hexane:isopropanol (96:4, v/v). ABA methyl ester was estimated by GC using an electron-capture detector (Harrison and Walton, 1975).

4. Radiomunnoassay (RIA)

The preparation of antibody to (RS)-ABA has been described (Walton et al., 1979). The assay mixture consisted of 10 μl [3H]ABA (115 pg, 19,000 d min$^{-1}$); 100 μl (S)-ABA, tissue extract, or solvent blank; 200 μl anti-ABA. All substituents were in 10 mM NaPO$_4$ pH 7.3. Standards and tissue extracts were run in triplicate.

The assay mixtures were incubated for 21 h in the dark at 4°C after which 100 μl of 0.5% human serum was added. The [3H]ABA-antibody complex was precipitated by the addition of 1 ml of a 75% saturated (NH$_4$)$_2$SO$_4$ solution. The tubes were centrifuged at 1,000 g for 15 min and 1 ml of the supernatant added to a 10 ml scintillation cocktail containing toluene: Triton X-100 (1:100) (US Biochemical Corp., Cleveland, OH, USA) (2:1, v/v) and 5 g l$^{-1}$ Omnifluor (New England Nuclear, Boston, MA, USA). Vials were held in the dark for 12 h at 4°C before counting. The standard curve was obtained by plotting dpm free [3H]ABA versus the logarithm of the concentration of unlabeled (S)-ABA added to the assay mixture. The curve was linear between 100 pg and 3.2 ng.

5. Chemicals

[3H]ABA (Walton et al., 1977b), 2-[14C]ABA (Sondheimer and Tinelli, 1971 b) and (S)-ABA (Sondheimer et al., 1971 a) were synthesized. PA and DPA were isolated from bean seed (Gillard and Walton, 1976).

Results

1. ABA Levels in Epidermal Strips

Table 1 indicates that ABA is present in the abaxial epidermal tissue of both T. gesneriana and C. communis and that its concentration rises when the leaves are subjected to a 10% fresh weight loss. In C. communis, there is an apparent 2-fold rise after 1.5 h while in T. gesneriana the rise is 3-fold after 4 h. In these experiments, ABA was estimated by GC.

Since our objectives were to compare the kinetics of ABA increase in epidermal tissue compared with the rest of the leaf, we attempted to measure ABA levels in both tissues after varying periods of stress. We had difficulties, however, in obtaining what we considered to be reliable results, particularly with the epidermal strips. Due to the relatively large number of samples required, it is necessary to keep the sample sizes small. In addition, we found that the extracts had to be purified extensively in order to remove compounds with retention times similar to that of ABA when we attempted to quantify by GC. Consequently, we often obtained large ABA losses during purification with resultant unreliable results. With the development of a RIA for ABA (Walton et al., 1979), we decided to use this technique since it is sensitive and requires relatively little purification. The results obtained in 2 kinetic experiments using RIA to estimate ABA are shown in Fig. 1A and B. In both experiments, there was a significant rise in ABA levels in the epidermis by 45 min after a 10% fresh weight

<table>
<thead>
<tr>
<th>Plant</th>
<th>Tissue</th>
<th>Unstressed</th>
<th>Stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. communis</td>
<td>epidermis</td>
<td>8</td>
<td>17$^b$</td>
</tr>
<tr>
<td></td>
<td>mesophyll</td>
<td>14</td>
<td>22$^b$</td>
</tr>
<tr>
<td>T. gesneriana</td>
<td>epidermis</td>
<td>12</td>
<td>35$^c$</td>
</tr>
<tr>
<td></td>
<td>mesophyll</td>
<td>15</td>
<td>100$^c$</td>
</tr>
</tbody>
</table>

$^a$ ABA levels determined by GC

$^b$ 1.5 h after stress

$^c$ 4 h after stress